A STUDY ON GENETIC POLYMORPHISM OF INTERLEUKIN-6 AND INTERLEUKIN-10 AND THEIR ASSOCIATION WITH CARDIOMETABOLIC RISK FACTORS IN HASHIMOTO'S THYROIDITIS

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Introduction:

Hashimoto's Thyroiditis (HT) is the most common organ specific autoimmune disease and the commonest cause of hypothyroidism. HT has a prevalence rate of 1-4% and found to be more common in women and increases with age [1,2]. HT is characterized by the presence of circulating thyroid antibodies and lymphocyte infiltration of the thyroid gland. Elevated circulating antibodies to thyroid antigens is seen in HT patients, of which anti-thyroid peroxidase antibodies (anti-TPO) being the most specific and sensitive for the diagnosis of the disease – positive in nearly 95% of patients; and anti-thyroglobulin antibodies (anti-Tg), positive in 60% - 70% of patients [1]. HT arises due to complex interactions between environmental and genetic factors. Studies have suggested a strong genetic influence on the development of autoimmune thyroid diseases [3]. Cytokines are key regulators of the immune and inflammatory responses and the genetic variants at cytokine encoding genes are potential risk factors for autoimmune thyroid disease. Several studies have reported, an elevated Interleukin – 6 (IL-6) levels and diminished Interleukin – 10 (IL-10) levels in the serum of HT patients [4]. Genetic polymorphism of IL-6 and IL-10, in their regulatory regions are implicated to influence the expression of protein and thereby contributes towards the risk of HT [5,6]. Although few studies have demonstrated the involvement of IL-6 and hs-CRP in HT pathogenesis, others have reported a contradictory outcome [7,8]. Though recent studies have reported the involvement of IL-10 in HT pathogenesis, it is still unclear, as whether IL-10 promotes or ameliorates the inflammation in HT patients [9]. A proper balance between proinflammatory cytokines (IL-6) and anti-inflammatory cytokines (IL-10) plays a critical role in the development of autoimmune diseases.

Many prospective studies have demonstrated the role of IL-6 and IL-10 cytokines in atherosclerotic lesion development, where their plasma levels are associated with genetic variants of interleukin genes IL-6 and IL-10, which in turn associated with risk of

cardiovascular risk [10]. HT is believed to exert obnoxious effects on cardiovascular system attributed to chronic inflammation [11]. Inflammation is implicated at all stages of progression of atherosclerosis. The chief inflammatory markers involved are IL-6 and high sensitivity – C Reactive Protein (hs-CRP) [7]. IL-6 is an important marker of endothelial dysfunction, which is regarded appropriately as an early step in the pathogenesis of atheroscelerosis [11]. IL-6 indirectly promotes atherogenesis by increasing the hepatic production of hs-CRP [8]. CRP is the major protein of the plasma, whose serum concentration can be directly related to magnitude of inflammation. IL-6 and hs-CRP are considered to be potential tools for predicting atherosclerosis and cardiovascular events [7].

Though, the role of cytokines in the HT pathogenesis has been investigated by various studies, there exists significant heterogeneity as well as ambiguity in their results. Only limited information is available about the cardiometabolic risk among the HT patients and their relationship remains unclear. A very few studies have been carried out, to explore the possible association between IL-6 and IL-10 cytokine gene polymorphisms and Hashimoto's thyroiditis in the Indian population. And also, the data about the association of cytokine gene polymorphism with the cardiometabolic risk in HT is very limited. Hence the present study is designed to investigate the role of genetic variants of IL-6 and IL-10 in the susceptibility of an individual to Hashimoto's Thyroiditis and their association with cardiometabolic risk factors in Hashimoto's Thyroiditis patients.

Aim:

- To determine the genetic polymorphism of Interleukin-6 (IL-6) and Interleukin-10 (IL-10) and their association with cardiometabolic risk factors in Hashimoto's Thyroiditis

Objectives:

- To determine the polymorphism of pro-inflammatory cytokine (IL-6) and anti-inflammatory cytokine (IL-10) genes in patients with Hashimoto's Thyroiditis.
- To identify the alleles in these patients associated with susceptibility to Hashimoto's Thyroiditis.
- To evaluate the circulating levels of the cytokines (IL-6, IL-10) in patients with Hashimoto's Thyroiditis.
- To assess the cardiometabolic risk factors (glycemic status, insulin resistance, atherogenic lipid profile, hsCRP, fibrinogen) in patients with Hashimoto's Thyroiditis.
- To find an association between genetic polymorphism of IL-6 and IL-10 genes and cardiometabolic risk factors in Hashimoto's Thyroiditis.

Research Question:

1) What is the role of IL-6 and IL-10 gene polymorphisms in the pathogenesis of Hashimoto's Thyroiditis in South Indian Population?

2) Is there any association of cytokine gene polymorphism with the cardiometabolic risk in Hashimoto's Thyroiditis?

Review of Literature:

Hashimoto's Thyroiditis (HT) is the most prevalent autoimmune thyroid disease, in which there will be intrathyroidal lymphocytic infiltration followed by gradual destruction of the thyroid gland leading to subclinical or overt hypothyroidism [3]. It is the most common cause of hypothyroidism and seen with a higher prevalence in females than males and increases with age [1,3]. The principal biomarkers for the diagnosis of HT are autoantibodies to thyroid peroxidase (TPO) antigen and thyroglobulin (Tg) antigen, present in the patients' sera. Anti-TPO antibodies and anti-Tg are found nearly in 90% and 80 % of HT patients respectively [3].

The etiology of HT is poorly understood and believed to arise due to complex interactions between environmental and genetic factors. Several genes were shown to be associated with HT disease progression and severity. Of that, genes for human leukocyte antigen, cytotoxic T lymphocyte antigen – 4, protein tyrosine phosphatase non-receptor- type 22, thyroglobulin, vitamin D receptor and cytokines are considered to be of utmost importance. The environmental factors such as excess iodine intake, infections, selenium deficiency, and certain drugs are implicated in the development of HT in genetically susceptible individuals [3].

In the pathogenesis of Autoimmune Thyroid Diseases (AITD) like HT, cytokines play a critical role, particularly in breaking autoimmune tolerance, autoantigen presentation, T and B lymphocyte activation, autoantibody production and inhibition of autoimmunity. Since cytokines are key regulators of the immune and inflammatory responses, any change in the expression of these cytokines are potential risk factors for the development of AITD. A thorough understanding of the role of a particular cytokine in the context of a specific immune response is essential for the development of appropriate strategies to modulate cytokine responses to maintain or restore health. The imbalance between proinflammatory and anti-inflammatory cytokines may play a role in the etiology of HT [12].

IL-6 is a proinflammatory cytokine, whose higher genetic producibility will affect the development of HT by promotion of inflammatory cytokines such as IFN-gamma and TNF-alpha and also by induction of differentiation of Th17 cells [6]. Nielsen CH et al have shown that Anti-TPO antibodies enhances the TPO elicited production of inflammatory cytokines like IL-6, TNF-alpha and IFN-gamma [13]. Another study has demonstrated that, the allele C in IL-6 -174 G/C was found to be associated with HT, which gives evidence of IL-6 having a proinflammatory activity and represents important facilitators of the immunologic process involved in HT as components of the autoimmune response. Single Nucleotide Polymorphism (SNP) of IL-6 gene at -572 C/G promoter region also showed an association with susceptibility to HT [14]. Another study done on Papillary Thyroid Cancer (PTC) patients with HT showed a significant increase in IL-4, IL-6, IL-9, IL-13 and IFN- gamma when compared to PTC patients without HT. In this study it is seen that autoimmune HT affects the cytokine profile of PTC patients by stimulating secretion of Th1 / Th2 / Th9 types of cytokines. IL10 is a Th2 cytokine that exerts anti-inflammatory effects by downregulating Th1 cytokines, TNF-alpha, IL1, IL8 and IFN-gamma. The CC genotype of IL-10 -592 gene is associated with higher producibility of IL-10 cytokines and seen more frequent in patients with severe HT than mild HT [15].

Hashimoto's Thyroiditis (HT) is the primary cause of noniatrogenic hypothyroidism and hypothyroidism has been observed to increase the risk for cardiovascular disease and atherosclerosis. In a Nationwide Cohort study done by Wei-Hung Chen et al, it was shown that HT increases the risk for CHD irrespective of baseline comorbidities including diabetes mellitus, hypertension, hyperlipidemia, stroke, chronic kidney disease. This suggests a mechanism independent of Hypothyroidism. Chronic inflammation in autoimmune diseases has been recognized as a contributing factor to CHD [16]. The results of this cohort study were supported by another study done by Mohammed N Atta et al, where they explained that HT is

an independent risk factor for cardiovascular diseases due to the low-grade inflammation seen in HT patients [17]. Chronic inflammation promotes the increase in inflammatory parameters and cytokines, with activation of vascular and endothelial cell dysfunction leading to decreased compliance of the vessel and the appearance of atheromatous plaque, independent of lipid profile alterations. Hence the present study was designed to investigate IL-6 and IL-10 gene polymorphisms which could serve as genetic markers for an individual's susceptibility to HT and correlate with severity of HT and cardiometabolic risk factors in patients with HT.

Materials and Methods:

Research committee and ethical clearance: The study was conducted after obtaining the institutional research and the ethical committee clearance.

Study Setting: This study was conducted in Department of Biochemistry in association with Department of Medicine, at Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

Study Design: Cross-sectional comparative study

Study Groups:

Group I: Age and gender matched healthy controls

Group II: Clinically and Biochemically diagnosed Hashimoto's thyroiditis patients

Study Participants: Clinically and biochemically diagnosed as Hashimoto's Thyroiditis patients visiting the Medicine outpatient department (OPD) in SMVMCH and who are willing for the study was included as study participants after obtaining informed consent.

Inclusion Criteria:

Controls (Group I): Age and gender matched healthy subjects were included in the study.

Cases (Group II): Subjects fulfilling the following criteria were included in the study:

- Subjects of age between 18 and 50 years
- Clinically proven symptoms of Hashimoto's Thyroiditis
- Elevated TSH with or without decreased T3 and T4
- Anti-TPO antibodies positive

Exclusion criteria:

- Known cases of Hyperthyroidism, Grave's disease, Thyroid cancer and Diabetes mellitus were excluded from the study
- Pregnant women or breast-feeding mother

Sample Size:

Group I **:** 120

Group II **:** 120

Sample size Calculation:

The sample size was computed using CaTS Power Calculator Software (Power Calculator for Genetic Studies, Center for Statistical Genetics, Michigan University, USA). The sample size was estimated at 5% level of significance with 80% power for the estimated disease prevalence of 4% [2] and minor allele frequency of 43.4% for IL-10 [-819 C/T] (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1800871).

Sample Collection:

Blood sample (5 ml of peripheral venous blood) was collected in EDTA vials with sterile technique after obtaining written consent from the participants included in the study. Plasma separated was used for cytokine and other biochemical parameter estimations. Genomic DNA was extracted from the whole blood by Kit method and was stored at -20°C till analysis.

Parameters studied:

Variables studies	Method
1) Anthropometric parameters	
Age	-
Body Mass Index (BMI	Calculated as Weight (Kg) / Height (m2)
Waist Hip Ratio (WHR)	Calculated using the formula Waist circumference/Hip circumference
Blood Pressure	Measured in sitting position using sphygmomanometer
2) Thyroid Profile	
fT3	CLIA
fT4	CLIA
TSH	CLIA
Anti Thyroperoxidase antibodies	ELISA
3) Glycemic status	
Fasting Blood Glucose	GOD-POD method

Fasting Insulin	ELISA
HOMA-IR	Fasting Insulin x Fasting Glucose / 405 (Fasting Glucose level in mg/dL)
4) Lipid Profile	
Total Cholesterol	Cholesterol oxidase method
Triglyceride	Glycerol kinase- peroxidase method
HDL-Cholesterol	Divalent cation precipitation method
LDL-Cholesterol	Calculated using Friedwald's formula [TC-(VLDL+HDL)]
VLDL	Calculated using the formula Triglyceride / 5
Atherogenic Index of Plasma	Calculated using the formula $\log_{10}$ (TG/HDL)
5) Inflammatory markers	
Interleukin-6	ELISA
Interleukin-10	ELISA
hs- CRP	ELISA
6) Genetic Polymorphism	
IL-6 (-174 G/C)	SfaNI - based PCR-RFLP
IL-6 (-572 G/C)	BsrBI - based PCR-RFLP
IL-10 (-592 C/A)	RsaI - based PCR-RFLP
IL-10 (-819 C/T)	MaeIII - based PCR-RFLP

Statistical Analysis:

Direct gene counting was carried out to identify the genotype frequencies in both cases and controls. The observed genotype frequencies in cases and controls were tested for Hardy-Weinberg Equilibrium (HWE) by Chi-square test. The difference in continuous variables which are normally distributed, was assessed using Student 't' test. For continuous variables which are non- normally distributed, Mann Whitney U test was used. To correlate different variables among the cases, Pearson and Spearman analysis was done. All statistical analysis

was carried out at 5% level of significance and p value <0.05 was considered significant. Statistical analysis was done using SPSS version 20.0.

Results:

A total of 240 subjects including 120 healthy controls (includes 117 females and 3 males) and 120 Hashimoto's Thyroiditis patients (includes 116 females and 4 males) were enrolled in this study. The mean age of Hashimoto's Thyroiditis subjects was found to be 31.26 ± 7.54, whereas, controls were found to have a mean age of 30.55 ± 7.44. On comparing the anthropometric and thyroid profile variables between cases and controls, there was a significant increase in the values of BMI, WHR, TSH and Anti-TPO antibodies among cases as compared to controls. A significant decrease in fT3 and fT4 was observed in cases versus controls.

In the present study, the difference in cardiometabolic factors like lipemic variables, fasting blood glucose, fasting insulin and HOMA-IR index was analysed between HT cases and healthy controls. A significant increase in the levels of fasting insulin, HOMA-IR, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) was observed. The risk ratios viz, TC/HDL, TG/HDL, LDL/HDL and atherogenic index of plasma (AIP) was also found to be increased in HT cases when compared to controls. When the inflammatory parameters were compared between HT cases and controls, pro-inflammatory factors like IL-6 and hs-CRP levels were found to be significantly increased in HT patients, whilst, there was significant decrease in the serum level of IL-10 in HT group when compared to controls.

Correlation analyses of TSH and Anti-TPO antibodies with other biochemical parameters were done. In correlation analysis, TSH was found to be negatively correlated with fT3, fT4 and a positive correlation was seen with Anti-TPO antibodies, IL-6, fasting insulin, HOMA-IR and LDL/HDL. Anti-TPO antibodies were also found to be negatively correlated with fT3, fT4 and positively correlate with TSH, IL-6, TC, LDL, TC/HDL, LDL/HDL, fasting insulin and HOMA-IR. When IL-6 was analysed for correlation with biochemical parameters, it was found

to negatively correlate with fT3 and fT4, whereas, positively correlate with TSH and Anti-TPO. On Linear Regression analysis, TSH and IL-6 were found to be independently associated with Anti-TPO antibodies.

Detection of Gene Polymorphism of IL-6 and IL-10

In the present study, the distribution of IL-6 (-174 G/C) genotypes were as follows; GG: 94 (78%), GC: 22 (18%), CC: 4 (3%) in HT cases and GG: 83 (69%), GC: 24 (20%), CC: 13 (11%) in controls. IL-6 (-174) CC homozygotes were found to decrease the odds of HT development when compared to GG homozygotes (OR: 0.27, CI: 0.06-0.92, p=0.03). With respect to allelic analysis of IL-6 (-174) gene, the OR for the risk of appearance of C allele compared to G allele, for the HT group was 0.54 (95% CI: 0.31-0.91, p = 0.01). No difference was seen with genotype and allele frequencies of other gene polymorphisms (IL-6 -572 G/C, IL-10 -592 C/A, IL-10 -819 C/T) studied.

Association of IL-6 and IL-10 gene polymorphisms with cardiometabolic factors among HT cases:

Table 1: Relationship of IL-6 (-174 G/C) polymorphism with biochemical variables among Hashimoto's Thyroiditis subjects

	GG (n=94)	**GC+CC (n=26)**	**'p' value**
TSH	23.8 (9.3 – 30.5)	11.1 (8.5 – 28.2)*	0.031
Anti-TPO	1300 (875 – 1425)	900 (642.5 – 1225)*	0.02
Total Cholesterol	207.3±19.9	198.08±18.4*	0.035
Fasting Blood Glucose	82.9±8.04	78.5±9.4*	0.019
HOMA-IR	3.5±1.02	3.08±0.66*	0.023

TSH : Thyroid Stimulating Hormone, Anti-TPO: Anti Thyroperoxidase antibody, HOMA-IR : Homeostatic Model Assessment of Insulin Resistance
The data are represented as mean ± S.D / Median (IQR)
P value calculated by Independent 't' test for normal distributed parameters and Mann-Whitney U test for Non-normal distributed parameters
** 'p' value < 0.05 is considered statistically significant*

Table 2: Relationship of IL-10 (-592 C/A) polymorphism with biochemical variables among Hashimoto's Thyroiditis subjects

	CC (n=64)	**CA+AA (n=56)**	**'p' Value**
Anti-TPO	1230 (900 – 1900)	993 (762 – 1300)*	0.028
IL-6	126.4±19.5	118.4±18.7*	0.025

Anti-TPO: Anti Thyroperoxidase antibody, IL-6: Interleukin 6
The data are represented as mean ± S.D / Median (IQR)
P value calculated by Independent 't' test for normal distributed parameters and Mann-Whitney U test for Non-normal distributed parameters
** 'p' value < 0.05 is considered statistically significant*

IL-6 (-572 G/C) and IL-10 (-819 C/T) gene polymorphisms were not found to associate with any of the cardiometabolic factors.

Discussion:

Hashimoto's Thyroiditis (HT) is an autoimmune disease, characterized by the destruction of thyroid cells by both cell- and antibody- mediated immune responses and considered the primary cause of non- iatrogenic hypothyroidism. Hypothyroidism has been observed to enhance the risk for cardiovascular disease and atherosclerosis, dyslipidemia and chronic inflammation being the associated risk factors. Despite the association between overt hypothyroidism and cardiovascular risk is a well-known fact, the role of thyroid autoimmunity in enhancing cardiovascular risk independent of thyroid function is still debatable. Few studies suggest a putative role of thyroid autoimmunity in atherosclerosis development independent of thyroid dysfunction. Chronic inflammation and endothelial dysfunction could be the link [11].

Hashimoto's Thyroiditis and Insulin resistance:

In the present study, fasting insulin as well as insulin resistance were found to be significantly increased in HT subjects when compared to healthy controls. And also, we found a positive association of TSH and Anti-TPO with Fasting Insulin and HOMA-IR, exhibiting the role of thyroid autoimmunity in insulin resistance. Mazaheri et al. in their study demonstrated a significant increase in fasting insulin only in HT subjects with highly positive Anti-TPO antibodies, whereas failed to demonstrate any difference in fasting Insulin and HOMA-IR between patients with and without HT [18]. Whereas, few studies have not shown any significant difference in insulin resistance between the HT patients and healthy controls [19]. The Anti-TPO antibodies by activating T helper 1 cells, increases the production of proinflammatory cytokines such as TNF-α and IL-6 causing inflammation, which in turn results in insulin resistance [13].

Hashimoto's Thyroiditis and Dyslipidemia:

A significant increase in the levels of TC, TG, LDL and VLDL was observed among HT cases when compared to controls, in our study. Also, our study demonstrated a positive correlation between Anti-TPO and TC, LDL. Tamer et al. have found an increased levels of TC, LDL-C, TG and non-HDL-C in HT subjects as compared to healthy controls. But the same study failed to display any difference in the levels of TC and LDL-C between overt hypothyroid and euthyroid patients with HT. In a previous research, despite, TSH not correlating with any lipid parameters, Anti-TPO antibodies were found to be positively correlating with TG levels in HT subjects, suggesting the association of thyroid autoimmunity with dyslipidemia, independent of thyroid function [20]. In contrast, Tagami et al., had observed a positive correlation between TSH and lipid levels [21]. Being an autoimmune disease, HT itself may be a risk factor for development of cardiovascular disease. As stated by Volpe's hypothesis, the helper T cells are not suppressed due to the prevalence of defective suppressor T cells in HT, leading into production of various cytokines such as IFN – γ, IL-2 and TNF– α which might cause hyperlipidemia [22]. Besides, our study also demonstrated an increase in Atherogenic Index of Plasma (AIP), surrogate marker of small dense LDL (sdLDL), in HT subjects when compared to healthy controls, which was considered to be a superior predictor of cardiovascular risk than the other lipid parameters [23].

Hashimoto's Thyroiditis and Inflammation:

The present study has witnessed a significant increase in the serum levels of proinflammatory markers such as IL-6 and hsCRP and a significant decrease in the anti-inflammatory cytokine, IL-10 in HT subjects when compared to controls. Though, IL-6 was found to correlate positively with both TSH and Anti-TPO, it was independently associated with Anti-TPO as shown by regression analysis. Present study agrees with the report given by Marchiori et al [7],

where an increase in IL-6 levels seen in HT subjects and gradually decreased after treatment. Another study done by Turemen et al, demonstrated an increase in the serum levels of IL-6, TNF-α and hs-CRP in HT patients. In their study, they exhibited a positive correlation between inflammatory markers and flow mediated dilation which displays, low-grade chronic inflammation as a factor to promote atherosclerosis in HT [24]. In a study done by Sieminska et al., [11], hypothyroid patients with positive Anti-TPO showed an increase in IL-6 level as compared to hypothyroid patients without Anti-TPO. Paradoxically, no association was found between IL-6 and Anti-TPO in HT subjects, in a study done by El-Shenawy et al [8]. IL-10 exerts its anti-inflammatory effect by inhibiting the synthesis of inflammatory cytokines, such as IL-1, tumour necrosis factor-alpha (TNF-α), IL-6 and Interferon-γ. We demonstrated a significant decrease in the serum levels of IL-10 in HT patients in comparison to controls, which is in agreement with the study done by De La Vega et al [25]. As said earlier, the imbalance between pro- and anti- inflammatory cytokines could be the reason for inflammation seen in HT. Albeit the IL-10 aids in preventing autoimmunity by counteracting with proinflammatory cytokines, its more evident only in the earlier stages of the disease. As the disease progresses, the effect of proinflammatory cytokine overrides the effect of anti-inflammatory cytokine [25].

Hashimoto's Thyroiditis and Cytokine gene polymorphism:

Previous studies have reported the association of cytokine gene polymorphisms with altered levels of cytokine production and susceptibility to various disorders. In our study, genetic polymorphism of IL-6 and IL-10 was selected, as the balance between proinflammatory and anti-inflammatory cytokines production influences the net outcome of an immune response. On analysis of distribution of genotype and allele frequencies of IL-6 gene (-174 G/C, -572 G/C) and IL-10 gene (-592 G/C, -819 G/C), a significant difference was seen with regard to CC homozygotes and C allele of IL-6 (-174 G/C) polymorphism in our study. While the

association of cytokine gene polymorphism with cardiometabolic factors was analyzed, there was a significant decrease in the serum levels of TSH, Anti-TPO, TC, FBG and HOMA-IR among C allele carrier of IL-6 (-174) gene when compared to GG homozygotes. Regarding IL-10 (-592 C/A) polymorphism, 'A' allele carrier found to have a significantly decreased levels of Anti-TPO and IL-6 as compared to CC homozygotes. However, no difference was found for IL-6 (-572 G/C) and IL-10 (-819 C/T) polymorphisms. While few studies demonstrated C allele carriers to increase the odds of developing HT, few reports observed increased frequency of G allele carriers among HT subjects [26]. Individuals with G allele, expressed higher plasma IL-6 levels when compared to carriers of C allele. Individuals with genetic predisposition to higher IL-6 secretion (G allele carrier) were observed to have higher triglyceride level, higher plasma FFA, lower HDL level and higher fasting insulin level [27]. Henningsson et al. found association between the IL6 CC genotype and low total cholesterol, LDL-C and triglycerides in women [28]. The present study is in agreement with the previous reports, where, a decrease in the levels of TSH, Anti-TPO, TC, FBG and HOMA-IR is seen in C allele carriers, as compared to IL-6 -174 GG homozygotes.

IL-10 is a pleiotropic cytokine that exerts its anti-inflammatory effects by downregulating the synthesis of pro-inflammatory cytokines. A putative role of IL-10 gene polymorphism on the susceptibility to AITD has been proposed. While Hunt et al [5] revealed no association of IL-10 polymorphism with autoimmune hypothyroidism, Inoue et al [15] has observed a significant association of IL-10 -592 CC genotype with susceptibility to HT and higher production of IL-10. In line with the study of Inoue et al [15], in the present study, though not significant, IL-10 (-592 C/A) variant allele carriers were found to decrease the odds of developing HT when compared to C allele. This statement was supported by the observation of significant reduction in the serum levels of IL-6 and Anti-TPO among IL-10 -592 A allele carriers in our study population. On the other hand, IL-10 (-819 C/T) polymorphism was neither significantly

associated with HT susceptibility nor with any of the cardiometabolic risk factors in the present study.

Conclusion:

The present study demonstrated a significant difference in the serum levels of proinflammatory and anti-inflammatory cytokines, influenced by their respective gene polymorphism. A chronic low-grade inflammation prevails in HT patients which has been attributed to lack of proper balance between proinflammatory and anti-inflammatory cytokines. This may lead to impending cardiovascular diseases. Therefore, assessment of inflammatory cytokines may help in attenuating the onset and progression of cardiovascular diseases in HT patients. Further elaborate studies dwelling into the molecular basis of immunopathogenesis of HT would provide greater and renewed insights into several important *hitherto* unknown facets. This could open newer vistas for the development of novel targeted therapeutics and reinforced personalised medicine for the management of Hashimoto's thyroiditis.

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